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# Chemical composition of hyphal walls of the ectomycorrhizal fungus *Cenococcum geophilum*

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## INTRODUCTION

The mycorrhizal association is more or less specific depending on the species of the fungus and the host (1). The fungal wall probably has a role in the identification mechanisms which lead to the formation of an ectomycorrhiza. A protein or glycoprotein in the fungal wall might associate with a molecule in the root cell wall (this hypothesis parallels what is known of identification mechanisms in other associations such as *Rhizobium*-legumes, pathogenic bacteria or fungi-plants, lichens).

Before isolation of surface molecules, it was necessary to determine the gross chemical composition of the wall and the location of the main polymers.

*Cenococcum geophilum* was chosen because of its very good growth in the fermentor. Its wall composition is still unknown. Furthermore, this mycorrhizal fungus has a very wide host spectrum. The results obtained could be used later as a model and a test for studies of other mycorrhizal fungi.

## EXPERIMENTAL WORK

- Wall preparation : *C. geophilum* was cultured in Pachlewski liquid medium, in a 19 litre-fermentor, for 9 days at 25°C. The mycelium was harvested, washed with distilled water, homogenized with glass beads in a MKS Braun homogenizer in the cold. Cell walls were again washed with distilled water then centrifuged at 4°C. 30 centrifugations, 20 min each, were effected with decreasing accelerations (from 10,000 to 3,000 g) ; this was necessary to eliminate all cytoplasmic material. Wall purity was

controlled with Transmission Electron Microscopy (T.E.M.) (Figure 1). Purified walls were then freeze-dried.

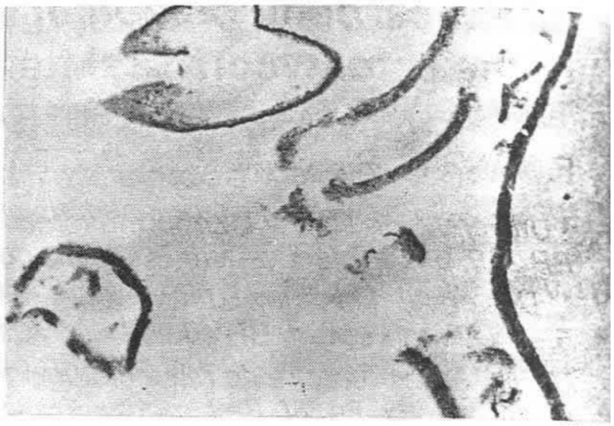


*Figure 1. Appearance of cell-wall*
Cell walls are totaly free of cytoplasmic material.

- Gross chemical composition of cell walls : the compositional studies effected on cell walls gave results reported in Tables 1 and 2. All hydrolysis were effected at 105°C in vacuum sealed tubes. Cinetics were necessary to obtain the best results.

*Table 1. Percentages of wall constituants in C. geophilum cell walls.*

| Components | % of dry matter | Assay method and preparative techniques |
|---|---|---|
| Carbohydrate | 43.1 | (phenol sulfuric after 3N HCl hydrolysis) (2) |
| Molar ratio : Gal/Man/Glc | 1/2.2/5.4 | (gaz chromatography Alditol acetates after 3N HCl hydrolysis) (3) |
| Amino acids | 8.6 | (D.N.F.B. after 6N HCl hydrolysis) (4) |
| Amino sugars | 3.6 | (Dimethyl benzaldehyde after 6N HCl hydrolysis) (4) |
| Uronic acid | 2.7 | (Metahydroxy diphenol after 2N $H_2SO_4$ hydrolysis) (5) |
| Phosphates | 0.36 | (Ammonium molybdate) (6) |
| Ashes | 8.8 | (gravimetric) |
| Ca++ | 0.032 | (Atomic absorption) |
| Mg++ | 0.077 | (Spectro) |
| Pigments | 11.3 | (Insoluble residue after hydrolysis) |
| TOTAL | 78.46 | |

Amino sugars are expressed in % of N-acetylglucosamine. The major part of amino sugars probably comes from acid hydrolysis of chitin, a $\beta$ (1-4) N-acetylglucosamine polymer.

*Table 2. Amino acid composition after HC1 hydrolysis*

| Amino acid | Mol % | μMole/g | | | | | |
|---|---|---|---|---|---|---|---|
| Neutral | 35.4 | Ala 54 | | Gly 63 | | Leu 40 | |
| | | IIe 18 | | Val 23 | | | |
| Dicarboxylic | 18.3 | Glu 50 | | Asp 63 | | | |
| Hydroxylated | 16.8 | Thr 50 | | Ser 53 | | | |
| Basic | 14.5 | Lys 34 | | Arg 36 | | His 12 | |
| Cyclic | 14.4 | Phe 20 | | Tyr 20 | | Pro 50 | |
| Sulfured | 0.6 | Cys 4 | | Met traces | | | |

Analysis by liquid chromatography in a Technicon autoanalyser after hydrolysis by 6N HCl for 10h.

- Fractionation of cell walls by ethylene diamine

Ethylene diamine is in wide use for yeast cell walls ; its advantage is to keep intact glycoproteic linkages which would be destroyed in the more usual KOH treatment (7-8). Treatment of walls with ethylene diamine gave 3 fractions (Table 3).

*Table 3. Percentages of fractions and chemical composition of the fractions :*

| Fraction | A<br>soluble in water<br>soluble in eth. diam. | B<br>insoluble in water:soluble in eth. diam. | C<br>insoluble in water and in eth. diam. |
|---|---|---|---|
| % of residue | 5.4 | 7.6 | 81.1 |
| Composition in % | | | |
| Carbohydrates | 79.2 | 57.6 | 38.8 |
| Amino acids | 19.2 | 16.1 | 2 |
| Amino sugars | - | traces | 3.2 |
| Phosphates | 0.12 | 0.14 | 0.07 |
| TOTAL | 98.52 | 73.8 | 44.07 |
| Insoluble residue after hydrolysis | - | +(pigments - proteins) | +(pigments + proteins + chitin) |

Pigments are complexed with chitin and protein. These black complexes are very resistant to acid hydrolysis. The C fraction insoluble in bases and containing the totality of chitin is the framework of the wall. The A and B fractions, soluble in bases are composed of glycoproteic polymers, some of which being located in the peripheral part of the cell. The molar ratio of sugars (Figure 2) indicates that fraction A is the richest in mannose, as compared to whole walls and to fractions B and C. On the other

hand, fractions B and C are very rich in glucose.

The Gal/Man molar ratio is identical in the A, B and C fractions and in the whole wall. This result corroborates the hypothesis of the presence of a same galactomannan in the totality of the wall.

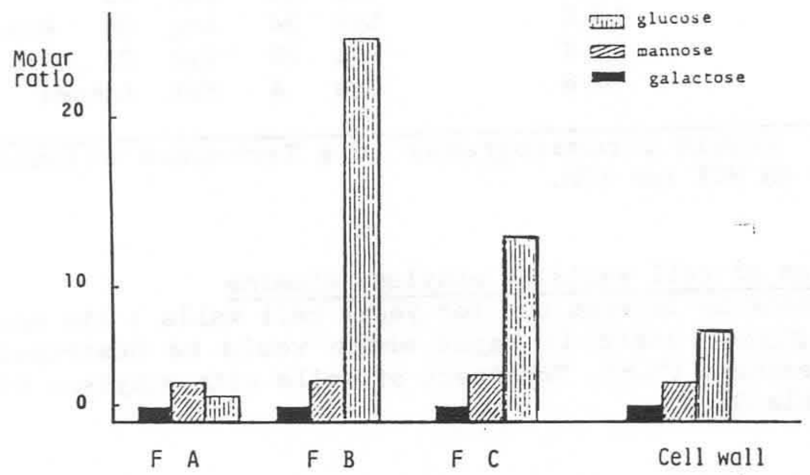


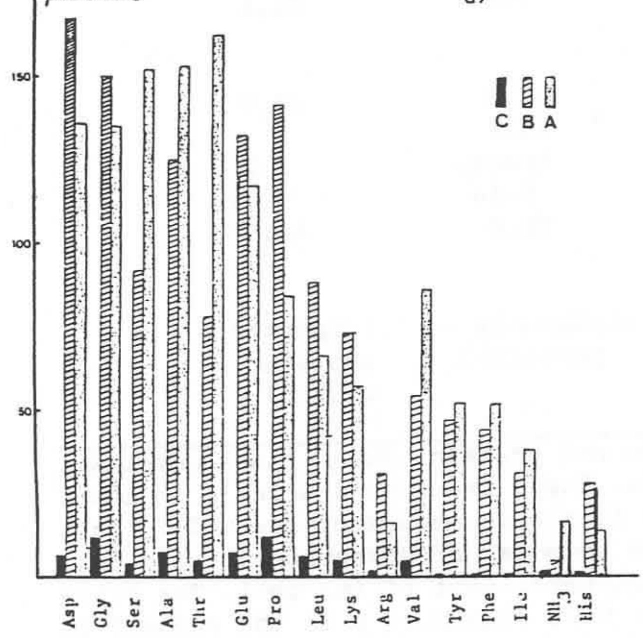

*Figure 2. Molar ratio of sugars in fractions A, B, C.*

The results of amino acid determination are reported in Table 4.

*Table 4. Amino acid composition*

Quantitative analysis of amino acids in fractions A – B and C.

a)




b)

molar %

| | Fractions | A | B | C |
|---|---|---|---|---|
| A.a. | Neutral | 36,5 | 30 | 41,4 |
| - | Dicarboxylic | 17 | 25 | 18,4 |
| - | Hydroxylated | 24 | 14 | 11,8 |
| - | Basic | 6,5 | 11 | 11,2 |
| - | Cyclic | 13,8 | 18,5 | 17,1 |

- Basic and dicarboxylic amino acids and proline are mainly present in fraction B.
- Hydroxylated amino acids are more abundant in fraction A.

- Molecular filtration of fraction A.

A preliminary purification of fraction A was effected by gel filtration on Tris acryl GF 2000 (IBF) (Fig. 3).

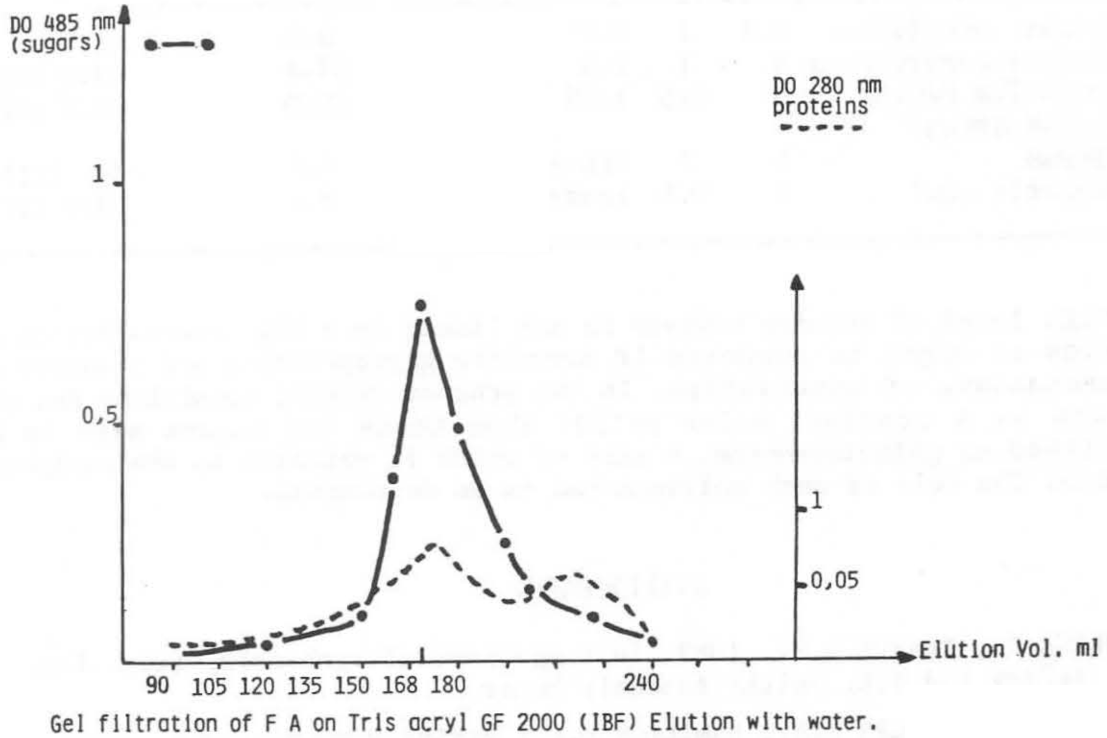



*Figure 3. Molecular filtration of fraction A.*

The concordance of a sugar peak with a protein peak eluted at 168 ml, evidences the presence of glycoproteins in fraction A. The apparent M.W. was estimated to be 150,000d. This peak, similar in its composition to the crude A fraction indicates that ethylene diamine allows the solubilization of peptidogalactoglucomannan.

## DISCUSSION

The results reported above on the chemical composition of the pigmented cell wall of *C. geophilum* lead to an hypothesis on the structure of this element in accordance with those proposed for other pigmented filamentous fungi. However comparative data reported in Table 5 reveal a higher proportion of mannose in *C. geophilum* cell wall than in other non mycorrhizal fungi.

*Table 5. Comparison of carbohydrate, amino acid and glucosamine contents between filamentous fungi.*

| Fungi | Molar ratio Glc/ | Gal/ | Man | Protein % | Amino sugar % |
|---|---|---|---|---|---|
| *Cenococcum geophilum* | 5.4 | 1 | 2.2 | 8.6 | 3.6 |
| *Dreschslera sorokiniana* | 5 | 1 | 1.1 | 17.4 | 9.6 (9) |
| *Sphaerostilbe repens* | 5 | 2.5 | 1.75 | 16.8 | 16.7 (10) |
| *Helminthosporium Spiciferum* | 5 | 1 | traces | 9.6 | 14 (11) |
| *Ceratocystis ulmi* | 5 | 0.2 | traces | 7.9 | 3.4 (12) |

The high level of mannose however is not linked to a high concentration of proteins as might be supposed if specific glycoproteins are involved in the mechanisms of association. In the present culture conditions Man and Gal are in a constant molar ratio, thus these two sugars seem to be associated as galactomannann, a part of which is situated in the periphery of cells. The role of such polymers has to be determined.

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